REMARKS

Claims 31-41 and 46-62 are pending in this application with claims 46 and 47 being withdrawn. After entry of this paper, claims 31-41 and 46-62 will remain pending and are presented for consideration.

Rejections Under 35 U.S.C. § 103(a)

Claims 31-41, 48-53, 56-60 and 62 stand rejected under 35 U.S.C. § 103(a) over Favaloro *et al.*, (Pathology, 25:152-158 (1993) ("Favaloro")) in view of Vischer *et al.* (Critical Reviews in Oncology/Hematology, 30:93-109 (1999) ("Vischer")), in view of Hoylaerts *et al.* (Biochem. J. 386:453-463 (1995) ("Hoylaerts")), and in view of Handin (U.S. Patent No. 5,321,127 ("Handin")). Claim 54 stands rejected under 35 U.S.C. § 103(a) further in view of Batz *et al.* (U.S. Patent No. 4,415,700 "Batz"). Claim 55 stands rejected under 35 U.S.C. § 103(a) further in view of Solen *et al.* (U.S. Patent No. 6,043,871 "Solen"). Claim 61 stands rejected under 35 U.S.C. § 103(a) further in view of Vicente (J. Biol. Chem., 263:18473-18479 (1988) ("Vicente")). Applicants traverse the rejections for the reasons set forth below.

Applicants' Claimed Invention

Applicants' claimed invention is directed to a method for detecting von-Willebrand's disease (vWD) by, *inter alia*, detecting a binding activity of von-Willebrand factor (vWF) in a sample, determining an amount of vWF-antigen in the sample, determining a ratio between the binding activity and the amount of vWF-antigen, comparing the ratio to a reference range, and detecting vWD based on the comparison.

According to Applicants' claimed invention, the binding activity detected is the binding activity of vWF in a sample to a soluble form or a portion of GP1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. This is a ***ristocetin cofactor activity assay***.

Prior to Applicants' claimed invention, no one had successfully used a soluble form or portion of GP1b(α), such as glycocalicin or any other soluble fragment of GP1b(α), to detect vWD based on the binding activity between vWF and the soluble fragment of GP1b(α) in a ristocetin cofactor activity assay. Applicants discovered for the first time that such a soluble

fragment can be successfully used to detect vWD using the method described and claimed in the present application.

Applicants' claimed invention provides a significantly improved ristocetin cofactor activity assay, meeting a long felt need in the art that has persisted over approximately the last 35 years, because Applicants' assay significantly reduces intra and interassay variability and demonstrates improved sensitivity to detect low levels of vWF over previously available assays.

Based on the arguments presented in the responses filed on February 22, 2007, November 20, 2007, which are incorporated by reference and for the reasons set forth below, Applicants respectfully submit that the claimed invention is not obvious and therefore is patentable.

The Examiner, at page 3-5 of the Office action, suggests that Favoloro teaches a method for detecting vWD according to Applicants' claimed invention except that Favaloro fails to teach the claimed step of detecting a binding activity of vWF in a sample to a soluble form or a portion of glycoprotein 1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. In particular, the Examiner asserts that Favoloro teaches a ***collagen binding assay*** that detects vWF activity in a sample (Office action, pg. 3, lines 16-17).

Given the deficiencies of Favoloro, the Examiner suggests that the teachings of Favoloro should be combined with the teachings of Vischer, Hoylaerts, and Handin to arrive at Applicants claimed invention. Applicants respectfully submit that none of Vischer, Hoylaerts, or Handin cures the deficiencies of Favoloro.

Favoloro, Vischer, and Hoylaerts

Vischer teaches that vWD is a heterogeneous disease having many types (see, *e.g.*, pg. 99, table 2). For example, Vischer teaches that Type 2 vWD refers to a qualitative vWF deficiency (pg. 99, table 2). Vischer further teaches that type 2 vWD has several subtypes. Vischer teaches that subtype-2B vWD can be distinguished from other type 2 variants because subtype-2B variants exhibit increased platelet agglutination at low concentrations of ristocetin, *i.e.*, the ***ristocetin-induced platelet aggregation assay*** (RIPA) (pg. 100, LH col.; pg. 103, LH col., para. 7.2.2.1; Table 4). The RIPA assay is a different assay than the ristocetin cofactor activity assay of Applicants' claimed invention in that the RIPA assay relies on the aggregation

of platelets from the patient plasma sample being tested, whereas the ristocetin cofactor activity assay introduces exogenous platelets *i.e.*, from a source other than the patient or, in the case of Applicants' claimed invention, a soluble form or a portion of glycoprotein 1b(α) not associated with a platelet into the assay (See Declaration of Pablo Bruguera, Ph.D., para. 5 ("Bruguera Declaration")).

Hoylaerts teaches the isolation of the GP1b protein from platelets (pg. 454, LH col., para. 2). Hoylaerts uses the isolated GP1b protein in an immunoassay to analyze how ristocetin mediates the binding of vWF to isolated GP1b (pg. 454, LH col.). Hoylaerts is deficient in that he does not teach a soluble form of GP1bα. Further, Hoylaerts is silent as to the isolated GP1b being used to make a diagnosis of vWD.

The Examiner suggests that Vischer provides a motivation to employ the method of Hoylaerts in addition to the collagen binding assay of Favoloro in order to differentiate subtype-2A and subtype-2B vWD (Office action, pg. 8, line 21-pg. 9, line 6). The Examiner suggests the two subtypes can be differentiated because Vischer teaches that subtype 2A is characterized by qualitative variants with decreased platelet-dependent function that is associated with the absence of molecular weight multimers and subtype-2B is characterized by qualitative variants with increased affinity for platelet GP1b (Office action, pg. 9). Applicants' respectfully disagree.

As mentioned previously, Vischer teaches that to differentiate subtype-2B from other type 2 von-Willebrand variants, one must perform a ***ristocetin-induced platelet aggregation assay*** (RIPA) (see pg. 103, LH col., paragraph 7.2.2.1.; Table 4). As taught by Vischer, the RIPA assay uses platelets from the patient being tested, *i.e.*, the RIPA assay tests aggregation of a patient's own platelets in the patient's plasma sample (see Bruguera declaration, para. 5). The platelets will aggregate in response to low concentrations of ristocetin, whereas type 2A and 2M will exhibit decreased aggregation and type 2N will exhibit normal aggregation (Table 4).

In contrast to the RIPA assay taught by Vischer, Hoylaerts teaches using GP1b fragments isolated from platelets (pg. 454, LH col.). Applicants submit that the method of Hoylaerts could not be used to differentiate subtype-2B from other type 2 variants of vWD, such as subtype-2A as asserted by the Examiner because it does not have all the necessary components required to

perform a RIPA assay, *i.e.*, it does not use the patient's own platelets from the patient's plasma sample being tested. Moreover, Hoylaerts is silent with respect to Hoylaert's method even being capable of diagnosing vWD, let alone differentiating among subtypes of type 2 vWD. Accordingly, Applicants submit that one skilled in the art would not look to the teachings of Favoloro and Vischer and combine them with Hoylaert's method in order to differentiate subtype 2A and 2B vWD because Hoylaert's does not teach a RIPA assay, the assay necessary according to Vischer to distinguish type 2B from other subtypes of type 2 vWD (see pg. 103, LH col., paragraph 7.2.2.1.; Table 4).

Applicants further submit that even if Hoylaert's taught a RIPA assay, such a teaching is irrelevant to Applicants' claimed invention as Applicants' claimed method does not require performing a RIPA assay. Rather, the binding activity detected in Applicants' claimed invention utilizes the ristocetin cofactor activity assay, *i.e.*, the binding activity of vWF in a sample to a soluble form of GP1bα that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. Because the claimed GP1bα fragment is not associated with the patient's own platelets, the claimed step cannot be a RIPA assay (Bruguera Declaration, paras. 4-5). Rather, vWF activity determined from agglutination in the presence of ristocetin and exogenous platelets (from a source other than the patient) or a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet is the *ristocetin cofactor activity assay* (see Bruguera Declaration., paras. 4-5). Accordingly, Applicants submit that the Examiner's combination of Favoloro, Vischer, and Hoylaert's fails to teach all elements of Applicants' claimed method, *i.e.*, a ristocetin cofactor activity assay, let alone a ristocetin cofactor activity assay using a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet.

Accordingly, for all the reasons discussed above, Applicants submit that the Examiner's asserted motivation for combining the teachings of Favoloro, Vischer, and Hoylaerts, namely to differentiate subtypes-2A and 2B vWD is flawed because the prior art teachings of Favoloro, Vischer, and Hoylaerts as combined by the Examiner do not actually permit distinguishing type 2A vWD and type 2B vWD from one another.

Further, Applicants submit that even if Hoylaerts method were a ristocetin cofactor activity assay according to Applicants' claimed invention, which Applicants' submit it is not, the

ristocetin cofactor activity assay cannot differentiate between type 2A and type 2B vWD because, as stated in the Declaration of Dr. Hans Deckmyn at paragraph 7, "the ristocetin cofactor assay [is] capable of detecting patients with subtypes 2A and 2B von Willebrand's disease." Accordingly, Applicants submit that there would be no motivation to combine the teachings of Favoloro, Vischer, and Hoylaerts based on the Examiner's rationale of differentiating type 2A and 2B vWD because such a differentiation cannot be made with a ristocetin cofactor activity assay.

In addition, the collagen binding assay of Favoloro which the Examiner combines with the teachings of Vischer and Hoylaerts likewise does not permit differentiation between type 2A and type 2B vWD because, as stated in the Declaration of Dr. Hans Deckmyn at paragraph 7, "the CBA [(collagen binding assay)] method…[is] capable of detecting patients with subtypes 2A and 2B von Willebrand's disease." Accordingly, using the collagen binding assay in conjunction with a ristocetin cofactor assay having a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet would not permit differentiation of type 2A from type 2B vWD because both the ristocetin cofactor assay and the collagen binding assay detect both the 2A and 2B vWD subtypes. For this reason, Applicants again submit that there would be no motivation to combine the teachings of Favoloro, Vischer, and Hoylaerts based on the Examiner's rationale of differentiating type 2A and 2B vWD because such a differentiation cannot be made with a ristocetin cofactor activity assay or the collagen binding assay.

The Examiner further supports the combination of Favoloro, Vischer, and Hoylaerts stating that "it has long been held that it is obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose" (Office action, pg. 11, citing *In re Kerkhoven*, 626 F.2d 846 at 850 (CCPA 1980)). Applicants submit that the Examiner's application of this case to the facts is incorrect.

Applicants submit that *In re Kerkhoven* stands for the proposition that "[i]t is *prima facie* obvious to combine two compositions each of which is known in the art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose." *In re Kerkhoven* at 850 (emphasis added). If the Examiner is asserting that the assays of Favoloro

(collagen binding assay) and Hoylaerts should be combined to create a third assay, such a combination is not supported by the rationale of *In re Kerkhoven.*

As stated therein, the combination of elements must be used for the very same purpose. Applicants submit that neither the assay of Favoloro or Hoylaerts is used for the very same purpose. For example, as asserted by the Examiner, Favoloro teaches a collagen binding assay (Office action, pgs. 9, lines 2-3). As stated in the Declaration of Dr. Hans Deckmyn, filed in this application on February 22, 2007, the collagen binding assay for detecting vWF "primarily involves the functional domain A3 of mature vWF" ("Deckmyn Declaration," pg. 3, paragraph 7b). In contrast, binding of vWF to the GP1b complex involves functional domain A1 of vWF (Deckmyn Declaration, pg. 3, paragraph 7b). Accordingly, the purposes of the collagen binding assay and an assay detecting vWF/GP1b binding are different, namely to detect defects in differing functional domains of vWF. In fact, Dr. Deckmyn indicates that whether the collagen binding assay or the ristocetin cofactor assay (which involves binding of vWF to the GP1b) is employed, "materially affects the diagnosis of von Willebrand disease" (Deckmyn Declaration, pg. 4, para. 8).

Further, Dr. Deckmyn states that "the CBA [(collagen binding assay)] and the ristocetin cofactor assay were <u>not</u> art recognized equivalents at the time [Applicants'] invention" (Deckmyn Declaration, pg. 4, para.4). As such, these assays cannot be combined under the rationale of *In re Kerkhoven* to form a third assay as the Examiner suggests because each of the individual assays cannot be used for the very same purpose.

In addition, even if there were a motivation to combine the teachings of Favoloro, Vischer, and Hoylaerts, which Applicants submit there is not, Applicants submit that a skilled artisan would have no reasonable expectation of success at differentiating type 2A vWD and type 2vWD using the methods of Favoloro and Hoylaerts. In particular, Hoylaerts is silent as to whether Hoylaerts' ELISA method for demonstrating the ristocetin mediated binding of vWF to GP1b can be used to diagnose vWD. Further, Hoylaerts provides no indication that the binding activity between vWF and Hoylaerts' GP1b in the absence of platelets would provide data sufficient for making a diagnosis of vWD. Accordingly, Applicants' fail to see how the method of Hoylaerts could be used to differentiate type 2A vWD and type 2vWD.

The Examiner has asserted at pg. 14-15 of the Office action that one of ordinary skill in the art "would have had a reasonable expectation of success in employing the method of Hoylaerts in place of platelet agglutination assays in order to detect type 2B vWD." Applicants submit that the method of Hoylaerts, if it could be used to detect vWD, could not be used to detect type 2B vWD in place of a platelet aggregation assay because, as was discussed previously, the assay as taught by Vischer that can distinguish type 2B vWD, the ristocetin-induced platelet aggregation assay (RIPA), requires the use of patient's own platelets (pg.103, LH col., para. 7.2.2.1; Table 4). In contrast, Hoylaerts' method does not use platelets from any source, but rather uses GP1b fragments isolated from platelets. Accordingly, Applicants fail to see how a skilled artisan would have a reasonable expectation of success at differentiating type 2A and 2B vWD based on the teachings of Hoylaerts.

Handin

The Examiner suggests that Handin teaches soluble GP1bα (Office action, pg. 11, lines 12-15). The Examiner suggests that it would have been obvious to one of ordinary skill in the art to use Handin's soluble GP1bα in Hoylaert's immunoassay in place of Hoylaert's GP1b protein because GP1b contains components that are allegedly not involved in the specific binding activity of vWF, while Handin's GP1bα allegedly contains the ristocetin-dependent vWF binding site.

Applicants' submit that a skilled artisan would have no motivation to use Handin's GP1bα fragment in Hoylaert's assay to arrive at Applicants' claimed method of detecting vWD because Handin does not teach measuring the binding activity of vWF in a sample to a soluble form of GP1b(α) that is not associated with a platelet. Rather, Handin teaches that soluble GP1b(α) fragments such as glycocalicin or rGp1bαQ221-L318 inhibit ristocetin-dependent binding of vWF to platelets and teaches an assay to demonstrate such inhibition ability of glycocalicin or rGp1bαQ221-L318. For example, as set forth in column 15, line 52, to column 16, line 3, Handin teaches: "The ability of recombinant GP1bα (rGP1bα) to inhibit ristocetin-dependent binding of [^{125}I]-vWF to platelets was assessed with *paraformaldehyde-fixed platelets*. . . . The ability of purified [glycocalicin] or the rGP1bα polypeptides to block vWF binding was

assessed by adding increasing concentrations of the appropriate test substance to the assay mixture" (emphasis added).

Therefore, Handin teaches a platelet aggregation assay that uses a soluble fragment of GP1b(α) such as glycocalicin or rGp1bαQ221-L318 to measure its ability to inhibit vWF binding to *platelets*, not to measure the binding activity of vWF in a sample to the soluble fragment of GP1b(α) that is not associated with a platelet. In fact, Handin does not teach any assay to detect the binding activity of vWF in a sample to a soluble fragment of GP1b(α). Moreover, Handin is silent with respect to the application of a soluble fragment of GP1b(α) to detect vWD.

Further, Applicants' submit that a skilled artisan would have no reasonable expectation of success in using any of Handin's soluble fragments of GP1b(α) in the assay of Hoylaert's to accurately detect the binding activity of vWF in a sample to a soluble form of GP1b(α). For example, Handin teaches that "[n]one of the recombinant polypeptides analyzed…contained the serine threonine-rich region to which O-linked oligosaccharides are attached" (col. 18, lines 45-47). In other words, Handin's recombinant GP1b(α) fragments were devoid of O-linked oligosaccharides. Given that a skilled artisan is aware that glycosylation patterns can be involved in protein-protein binding, Applicants submit that the absence of O-linked oligosaccharides from Handin's recombinant GP1b(α) fragments would preclude a skilled artisan from having a reasonable expectation of success at using those fragments to obtain appropriate levels of binding to vWF necessary for a diagnosis of vWD.

Even though Handin teaches that the biologic activity of rGpIbαL318, one recombinant GP1b(α) fragment, had the same biological activity as glycocalicin, a larger, glycosylated proteolytic fragment of GP1b, Handin measured the biological activity of the fragment by inhibition of ristocetin-dependent binding of vWF to *platelets*, not by determining the binding activity of vWF to the soluble GP1b(α) fragment (col. 18, lines 48-52). Accordingly, Applicants' submit that Handin's observation regarding the biologic activity of rGpIbαL318 is irrelevant to the effects of those glycosylation patterns on the binding activity of vWF to soluble GP1b(α) as useful for an assay for detecting vWD.

Accordingly, Applicants submit that even if a skilled artisan had a motivation to use a soluble fragment of GP1b(α) of Handin in the assay of Hoylaerts, a skilled artisan would not

have a reasonable expectation that using any of the recombinant GP1b(α) fragments of Handin would produce the requisite biological activity to provide an accurate measurement of vWF binding activity in the sample necessary for making a correct diagnosis of vWD.

Moreover, Handin provides no indication that the binding activity between vWF and Handin's GP1b(α) fragments in the absence of platelets would provide data sufficient for making a diagnosis of vWD. Accordingly, Applicants submit that a skilled artisan would have no reasonable expectation that using any of the recombinant GP1b(α) fragments of Handin would produce the requisite biological activity to provide an accurate measurement of vWF binding activity in the sample necessary for making a correct diagnosis of vWD.

In addition, Applicants submit that a skilled artisan would have no motivation or reasonable expectation of success in using the soluble GP1b(α) fragments of Handin to replace GP1b in the method taught by Hoylaerts because the prior art, discussed below, indicates that the binding activity between vWF and soluble GP1b(α) is not robust enough to provide clinically relevant test data to allow accurate discrimination between normal samples and samples from patients with vWD.

Christophe, cited by the Examiner in the October 23, 2006, Office action, addresses this point. Christophe compared the binding capacity of plasma vWF from type 2 (*i.e.*, type II) vWD patients and normal controls to a soluble fragment of GP1b(α) glycocalicin and to platelet GP1b (see, Christophe, pg. 3554, LH col.). Christophe found that, while ristocetin-induced binding of plasma vWF to fixed platelets correlated with the clinical phenotypes of type 2 vWD, the binding of plasma vWF from type 2 vWD patients to glycocalicin is normal (see, Christophe, Figure 6, Table 1 and pg. 3557, RH col., and pg. 3560, LH col.). In other words, Christophe disclosed that the binding activity between vWF in a plasma sample and a soluble fragment of GP1b(α) glycocalicin detected in its experiment did not provide clinically relevant data to allow discrimination between normal samples and samples from patients with vWD. Therefore, one of skill in the art in view of the teachings in Christophe would have been discouraged from using a soluble fragment of GP1b(α), such as fragments taught by Handin, instead of platelets to detect vWD. One of skill in the art in view of the teachings in Christophe also would not have expected

that a soluble fragment of GP1b(α) can be successfully used to detect vWD based on its binding activity to vWF in a sample.

Batz, Solen, and Vicente

Similarly, none of the other references cited by the Examiner teach or suggest that a soluble fragment of GP1b(α) can be successfully used to detect vWD based on its binding activity to vWF in a sample. As discussed previously, the Examiner asserts that Favaloro teaches a method for detecting vWD using a collagen-binding assay (Office action, pg. 6, lines 16-17). Hoylaerts teaches use of an immunoassay to study how ristocetin mediates the binding of vWF to the GP1b complex (*see, e.g.*, Hoylaerts, pg. 454, LH col., first para.). Batz teaches use of hydrophilic latex particles as carrier materials for biological and/or immunologically active substances in diagnostic agents (*see, e.g.*, Batz, abstract and the first paragraph in detailed description). Solen teaches a system and a method for measuring the platelet aggregation in whole blood in response to standard aggregating agents (*see, e.g.*, Solen, abstract). Vicente teaches a 45 KDa GP1b(α) N-terminal fragment of GP1b(α) (glycocalicin) that is capable of interacting with purified surface-bound vWF (*see, e.g.*, Vicente, abstract, pg. 18475, LH col.). Therefore, not one of the cited references teach or suggest that a soluble fragment of GP1b(α) can be successfully used to detect vWD based on its binding activity to vWF in a sample.

For all these reasons, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of the pending claims under 35 U.S.C. 103(a).

Long-Felt Need

Even if there is a motivation and reasonable expectation of success to combine the teachings of Favoloro, Vischer, Hoylaerts, Handin, Batz, Solen, and Vicente, as suggested by the Examiner, Applicants submit that Applicants' invention is not obvious because Applicants' invention satisfies a long felt need in the art for an improved ristocetin cofactor activity assay for diagnosing vWD.

Applicants submit that since the development of the ristocetin cofactor activity assay in the early 1970s, those skilled in the art of diagnosing vWD recognized several deficiencies in the accuracy, sensitivity, and reproducibility of the assay (see Bruguera declaration, paras. 6-7).

Even as recently as 2007, Favoloro II (Favoloro, (2007), *Seminars in Thrombosis and Hemostasis*, 33(8):727-744) acknowledged that "over the subsequent 35 years or so [since the assay was first introduced], several significant limitations to the ristocetin cofactor assay have emerged" (Favoloro II, pg. 729, RH col.). In particular, as outlined in Favoloro II and described in the Declaration of Pablo Bruguera at paragraphs 6-7, the traditional ristocetin cofactor activity assay has suffered from poor interassay variability, poor intraassay variability, poor reproducibility, and low sensitivity. In particular, the classical ristocetin cofactor activity assay "cannot reliably provide an estimate of von Willebrand factor below around 20%...[which] is a serious limitation" (Favoloro II, pg. 730, LH col.). As of the priority date of this application, namely July 5, 1999, these deficiencies were recognized in the art (see Bruguera declaration, para. 7). In particular, as stated by Dr. Bruguera in his declaration:

> "[t]here has been a long-felt need in the field of von Willebrand testing and diagnosis since the time the ristocetin cofactor activity assay was first developed for an improved ristocetin cofactor activity assay that significantly reduces or eliminates intra and interassay variability and improves sensitivity for measuring von Willebrand factor, *i.e.*, an assay that can measure the low levels of von Willebrand factor characteristic of severe von Willebrand disease subtypes with levels of von Willebrand factor below 20%" (Bruguera declaration, para. 8).

For these reasons, Applicants submit that there was as of the priority date of this application, a recognized and persistent need in the art for an improved ristocetin co-factor assay that addresses these limitations.

Applicants submit that others had not solved the need for an improved ristocetin cofactor assay as of the priority date of this application. In particular, while others were attempting to improve the classical ristocetin cofactor assay at least as of the priority date of this application, "none has been as successful in correcting he deficiencies of the classical ristocetin cofactor activity assay's lack of reproducibility and high inter assay variability as the Applicants' claimed assay" (Bruguera declaration, para. 9). For example, the Dade Behring ristocetin cofactor assay, available as early as January 1999 (see "Exhibit C" of Bruguera declaration), may have improved interassay and intraassay variability due to performance of the assay in an automated system. However, "even automated assays, such as the Dade Behring assay…have not fully

addressed all the drawback of the classical ristocetin cofactor assay" (Bruguera declaration, para. 10). Accordingly, "further improvements in interassay and intrassay variability were still necessary as of the priority date" (Bruguera declaration, para. 10).

In contrast, Applicants' claimed assay performed on an automated system provides results that are "more precise than what is seen with the Dade Behring ristocetin cofactor assay" (Bruguera declaration, para. 12). For example, as stated in the declaration of Pablo Bruguera at paragraph 18, Applicants' claimed assay can provide detection levels at least as low as 10% vWF, overcoming the "serious limitation" of the classical assay acknowledged by Favoloro II (Favoloro II, pg. 730, LH col.; see Bruguera declaration, para. 18).

Further, Applicants' claimed invention results in coefficients of variation for results of samples tested that are significantly lower than Dade Behring's coefficients of variation for its ristocetin cofactor assay. For example, Applicants' claimed assay has 1.8-2.0 times less within run variation for pathological controls than the Dade Behring assay when compared to Dade Behring's lower range of the within run coefficient of variation of pathological controls. Applicants' claimed assay had 1.9-2.1 times less total variation for pathological controls than the Dade Behring assay when compared to Dade Behring's lower range of total coefficient of variation for pathological controls. When comparing normal controls, Applicants' claimed assay had 1.6 times less within run variation than the lower range of the within run coefficient of variation for Dade Behring's normal controls. Applicants' claimed assay also had 1.5 times less total variation than the lower range of the total coefficient of variation for Dade Behring's normal controls. (see Bruguera declaration, para. 16). According to Dr. Bruguera's declaration, these improvements can be attributed to the use of the soluble form or portion of the glycoprotein 1b(α) that is not associated with a platelet rather than to automation of the assay (para. 15).

Given the lower coefficients of variation resulting from Applicants' claimed invention, the mean % vWF activity detected across samples using Applicants' claimed invention is more accurate. Accordingly, when a ratio is made for the value for vWF activity with the amount of von-Willebrand antigen as required by Applicants' claimed invention, the ratio will be more accurate. Accordingly, a more accurate diagnosis can be made. (see Bruguera declaration, para. 17).

Applicants further submit that the patentability of Applicants' claimed invention is evidenced by the fact that in all the years intervening since the development of the ristocetin cofactor assay, no one has attempted to improve the ristocetin cofactor assay by using a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet. If such an improvement were so obvious, Applicants respectfully question why no one else had done so prior to Applicants' priority date.

Accordingly, for all these reasons, Applicants' respectfully submit that Applicants' claimed invention meets a long felt need in the art for an improved ristocetin cofactor activity assay. This conclusion is supported by Dr. Bruguera in his declaration at paragraph 19 where he states that "the improvements demonstrated by Applicants' claimed ristocetin cofactor assay meet the long felt need that has persisted in the art for the last 35 years or so for an assay that addresses the concerns expressed by Favoloro [II][,]" namely concerns related to variability, reproducibility, and sensitivity of the classical assay (see Favoloro II, pg. 729, RH col-pg. 730, LH col.).

For all these reasons, Applicants respectfully request that the Examiner reconsider and withdrawn the rejection of the pending claims under 35 U.S.C. 103(a) on the basis that the claimed invention meets a long-felt need in the art for an improved ristocetin cofactor activity assay.

Unexpected Results

Applicants submit that Applicants' claimed invention is also not obvious in view of the unexpected results that Applicants' assay obtains over the prior art. In particular, Applicants' assay requires the step of detecting a binding activity of vWF in a sample to a soluble form or a portion of GP1b(α) that is not associated with a platelet in the presence of ristocetin or a functionally equivalent substance. This step is a ristocetin cofactor activity assay (see Bruguera declaration, paras. 4-5).

The classical ristocetin cofactor activity assay has been available since the early 1970s (Favoloro II, pg. 720, RH col.). However, as discussed above, the classical ristocetin cofactor activity assay has suffered from poor intraassay reproducibility, high interassay variability, and high interlaboratory variability. Further, the classical ristocetin cofactor activity assay has

proven unreliable at estimating vWF below around 20% (100% being normal) (Favoloro II, pg. 730, LH col., lines 3-14).

Given the problems with the traditional ristocetin cofactor activity assay, Applicants believe that the step of the claimed invention which tests the ristocetin cofactor activity shows unexpected results.

Applicants' performed step "(a)" of Applicants' assay as recited in claim 1 on an automated instrument, the ACL Top (Instrumentation Laboratory, Lexington, MA) with control plasma samples, ristocetin, and the GP1b(α) fragment. Five replicates of the each control plasma sample were processed for each of three controls: a normal control plasma (NC), a borderline vWF deficiency plasma (CL1), and a vWF deficient plasma (CL2). Controls were assessed using 5 replicates of each control run twice a day for 5 days, resulting in 50 replicates for each control. The within run coefficient of variation for CL1 was 3.4%, for CL2 was 3.0%, and for NC was 4.9%. The total coefficient of variation (all replicates) for CL1 was 3.6%, for CL2 was 4.0%, and for NC was 5.4%. (see Bruguera declaration, paras. 13-14).

In contrast, an automated ristocetin cofactor activity assay from Dade Behring (see Exhibit C of Bruguera declaration) demonstrates results from an automated assay performed with ristocetin, stabilized platelets, and control plasma. As outlined in the Dade Behring pamphlet (see Exhibit C of Bruguera declaration), 8 replicates of each of a pathological control plasma and a normal control plasma were assayed in one run per day for 5 days. For normal control plasma, the within-run precision (or coefficient of variation) was between 8.0 and 9.6%, while the total precision ranged from 8.0 to 10.3%. For the pathological control plasma, the within-run precision ranged from 6.1 to 16.2%, while the total precision ranged from 7.6 to 16.9% (see Exhibit C of Bruguera Declaration and Bruguera Declaration, para. 14).

As discussed above with respect to "long-felt need," the data from Applicants' claimed invention show a significant and valuable improvement over the Dade Behring assay (Bruguera declaration, para. 15). For example, Applicants' claimed assay has 1.8-2.0 times less within run variation for pathological controls than the Dade Behring assay when compared to Dade Behring's lower range of the within run coefficient of variation of pathological controls. Applicants' claimed assay had 1.9-2.1 times less total variation for pathological controls than the

Dade Behring assay when compared to Dade Behring's lower range of total coefficient of variation for pathological controls. When comparing normal controls, Applicants' claimed assay had 1.6 times less within run variation than the lower range of the within run coefficient of variation for Dade Behring's normal controls. Applicants' claimed assay also had 1.5 times less total variation than the lower range of the total coefficient of variation for Dade Behring's normal controls. (see Bruguera declaration, para. 16).

Further, as stated in the declaration of Dr. Bruguera at paragraph 18, the assay according to the invention has a sensitivity able to detect at least 10% vWF. Not being able to detect below 20% vWF is one of the disadvantages Favoloro II points out in his paper. At the time of writing his paper in 2007, according to Favoloro, this problem had not yet been solved. That Applicants' have created an assay that can detect below 20% vWF should therefore be seen as a significant and valuable improvement over the classical ristocetin cofactor activity assay (Bruguera declaration, para. 18).

These improved results provide a significant practical advantage over the prior art because with reduced variation, the von Willebrand activity detected is more accurate. Accordingly, when a ratio is made for the value for von Willebrand activity and the von-Willebrand antigen amount determined in step (b) of Applicants' claimed invention, the ratio will be more accurate and the ultimate diagnosis will be more accurate. (see Bruguera declaration, para. 17).

For all these reasons, Applicants respectfully request that the Examiner reconsider and withdrawn the rejection of the pending claims under 35 U.S.C. 103(a) on the basis that Applicants' claimed assay provides unexpected results and a significant practical advantage over the prior art.

Despite the fact that it had been known since 1988 that a soluble fragment of GP1b(α) glycocalicin is capable of binding to vWF, no one had successfully used glycocalicin or any other soluble fragment of GP1b(α) to detect vWD based on the binding activity between the vWF and the soluble fragment of GP1b(α) prior to Applicants' invention. Applicants discovered for the first time that such a soluble fragment can be successfully used to detect vWD using the method described and claimed in the present application. Accordingly, Applicants submit that

their invention for a method for detecting vWD using a soluble fragment of GP1b(α) is not obvious for all the reasons stated herein.

Applicants therefore respectfully submit that claim 31 and claims 32-41 and 48-62 dependent therefrom are novel and non-obvious over Favaloro, Vischer, Hoylaerts, Handin, Batz, Solen, and Vincent for all the foregoing reasons. Applicants therefore respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a).

CONCLUSION

Applicants believe that the pending claims are now in condition for allowance. The Examiner is invited to telephone the undersigned attorney to discuss any remaining issues. Early and favorable actions are respectfully solicited.

Respectfully submitted,

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